

University of Massachusetts Medical School

eScholarship@UMMS

GSBS Student Publications

Graduate School of Biomedical Sciences

2010-01-22

Lineage-specific effects of Notch/Numb signaling in post-embryonic development of the Drosophila brain

Suewei Lin

University of Massachusetts Medical School

Et al.

Let us know how access to this document benefits you.

Follow this and additional works at: https://escholarship.umassmed.edu/gsbs_sp



Part of the [Neuroscience and Neurobiology Commons](#)

Repository Citation

Lin S, Lai S, Yu H, Chihara T, Luo L, Lee T. (2010). Lineage-specific effects of Notch/Numb signaling in post-embryonic development of the Drosophila brain. GSBS Student Publications. <https://doi.org/10.1242/dev.041699>. Retrieved from https://escholarship.umassmed.edu/gsbs_sp/1696

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in GSBS Student Publications by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.

Lineage-specific effects of Notch/Numb signaling in post-embryonic development of the *Drosophila* brain

Suewei Lin^{1,*}, Sen-Lin Lai^{1,*†}, Huang-Hsiang Yu^{2,*}, Takahiro Chihara^{3,‡}, Liqun Luo³ and Tzumin Lee^{1,2,§}

SUMMARY

Numb can antagonize Notch signaling to diversify the fates of sister cells. We report here that paired sister cells acquire different fates in all three *Drosophila* neuronal lineages that make diverse types of antennal lobe projection neurons (PNs). Only one in each pair of postmitotic neurons survives into the adult stage in both anterodorsal (ad) and ventral (v) PN lineages. Notably, Notch signaling specifies the PN fate in the vPN lineage but promotes programmed cell death in the missing siblings in the adPN lineage. In addition, Notch/Numb-mediated binary sibling fates underlie the production of PNs and local interneurons from common precursors in the IAL lineage. Furthermore, Numb is needed in the lateral but not adPN or vPN lineages to prevent the appearance of ectopic neuroblasts and to ensure proper self-renewal of neural progenitors. These lineage-specific outputs of Notch/Numb signaling show that a universal mechanism of binary fate decision can be utilized to govern diverse neural sibling differentiations.

Key words: Notch, Numb, Brain, *Drosophila*, Postembryonic, Antennal lobe projection neurons, Apoptosis

INTRODUCTION

The remarkable diversity of cell types in the *Drosophila* nervous system develops in a stepwise fashion. First, neural progenitors, characterized by different transcriptional codes, emerge during neuroectoderm patterning (Urbach and Technau, 2003). Conserved mechanisms underlie the development of mammalian nervous systems (Briscoe et al., 2000). Second, these progenitors produce multiple neuron types often in an invariant sequence through self-renewing asymmetric divisions (Pearson and Doe, 2004; Yu and Lee, 2007). Each self-renewing asymmetric division regenerates the progenitor while depositing an intermediate precursor that may divide into two postmitotic neurons. Third, molecular asymmetries during the neuron-producing mitoses provide different fates to sister neurons (Bardin et al., 2004; Kimura et al., 2008). This step involves coordination between the localization of cell-fate determinants and the orientation of the plane of cell division (Knoblich, 2008). The binary sibling fate decision is generally referred to as asymmetric cell division, although such divisions may not be morphologically asymmetrical (Buescher et al., 1998). Thus, a common thread in neuronal diversification is the generation of two distinct cells from a precursor.

Studies of *Drosophila* central brain lineages indicate that neuron fate depends on lineage and birth timing (Jefferis et al., 2001; Yu and Lee, 2007). But the evidence for fate diversification during final mitoses is lacking. In the *Drosophila* mushroom bodies (MBs), five types of MB neurons are sequentially derived from common

progenitors in a non-overlapping manner (Lee et al., 1999; Zhu et al., 2003). There is no evidence for fate diversification during final neuron-producing mitoses, which should otherwise yield two neuron types at one time.

In the antennal lobes (ALs) of *Drosophila* central brain, many types of uniglomerular projection neurons (PNs), which relay olfactory information from the peripheral olfactory receptor neurons (ORNs) to the MBs and the lateral horns (LHs), can be distinguished based on their innervation of different AL glomeruli as well as the acquisition of different stereotyped patterns of axon arborization in the LHs (Jefferis et al., 2001; Marin et al., 2002; Wong et al., 2002). Notably, they arise from three AL neuroblasts (Nbs); specific Nbs make specific PN types (Jefferis et al., 2001). Birthdating of individual PN types in the anterodorsal PN lineage reveals derivation of distinct neuron types in an invariant non-overlapping temporal sequence (Jefferis et al., 2001). As this PN lineage consists only of uniglomerular PNs (Jefferis et al., 2001; Lai et al., 2008), the generation of one uniglomerular PN type at one time again provides no evidence for neuronal diversification during final neuron-producing mitoses. By contrast, the lateral lineage, which includes diverse types of PNs as well as various non-PNs, may produce PNs and non-PNs in the same window (Lai et al., 2008). However, it remains to be determined in the lateral lineage if distinct postmitotic neurons are generated concurrently through asymmetric cell divisions.

A conserved mechanism underlies cell diversification through asymmetric cell division in diverse contexts (Bardin et al., 2004). It involves asymmetric localization of the membrane-associated protein, Numb, during mitosis, resulting in inheritance of Numb by only one of the two daughter cells (Knoblich et al., 1995; Rhyu et al., 1994; Spana and Doe, 1995; Spana and Doe, 1996; Spana et al., 1995). Presence of Numb silences Notch signaling, which otherwise takes place in both siblings, as Notch and its ligand Delta exist broadly (Guo et al., 1996; Zeng et al., 1998). Numb antagonizes Notch through promoting endocytosis of Sanpodo, a four-pass transmembrane protein with expression on the cell surface that is essential for activation of Notch by Delta (Hutterer and Knoblich, 2005; O'Connor-Giles and Skeath, 2003). Asymmetric cell division

¹Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA 01605, USA. ²Howard Hughes Medical Institute, Janelia Farm Research Campus, Ashburn, VA 20147, USA. ³Howard Hughes Medical Institute, Department of Biology, Stanford University, Stanford, CA 94305, USA.

*These authors contributed equally to this work

[†]Present address: Howard Hughes Medical Institute, Institutes of Neuroscience and Molecular Biology, University of Oregon, Eugene, OR 97403, USA

[‡]Present address: Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, 113-0033 and CREST, JST, Japan

[§]Author for correspondence (leet@janelia.hhmi.org)

thus gives rise to one Notch-on and one Notch-off cell. Notch is a large transmembrane receptor, which is proteolytically cleaved after binding with Delta (Schweisguth, 2004). After cleavage, the Notch intracellular domain translocates into the nucleus, where it can modulate gene expression to dictate cell fate (Bailey and Posakony, 1995; Lecourtis and Schweisguth, 1998; Struhl and Adachi, 1998). Consistent with this mechanism, loss of Notch versus Numb causes reciprocal cell-fate transformation in sister cells derived after asymmetric cell division (e.g. Skeath and Doe, 1998). Its broad involvement in controlling diverse opposing cell fates further implicates a common Notch-dependent mechanism as a trigger to activate cell differentiation along one rather than the other pre-programmed path following each asymmetric cell division.

In *Drosophila*, Notch/Numb-dependent asymmetric divisions underlie cellular diversification in the development of various neural structures, including the embryonic ventral ganglion (Karcavich and Doe, 2005; Skeath and Doe, 1998; Wheeler et al., 2008), the external sensory organs (Jan and Jan, 2001) and the ORN lineages (Endo et al., 2007). However, non-self-renewing asymmetric cell division has not been shown in the developing *Drosophila* brain; and the roles of Notch and Numb in the derivation of the enormous cell diversity in the *Drosophila* central brain remain undetermined.

Here we revisited the three AL PN lineages (referred to as adPN, IAL and vPN) and show that Notch/Numb-mediated asymmetric cell division governs the development of all three lineages in the *Drosophila* central brain. Paired sister cells acquire different fates. Notably, only one in each pair of postmitotic neurons survives into the adult stage in both adPN and vPN lineages. And Notch signaling promotes PN fate in the vPN lineage but specifies the mysterious sibling fate in the adPN lineage. By contrast, Notch/Numb-mediated asymmetric cell division underlies the derivation of PNs and local interneurons from common precursors in the IAL lineage. Furthermore, Numb is needed in certain lineages (e.g. IAL but not adPN or vPN) for preventing appearance of ectopic Nbs to ensure proper self-renewal of neural progenitors. A universal Notch/Numb-dependent mechanism of binary fate decision may govern diverse neural sibling differentiations based on the origins.

MATERIALS AND METHODS

Fly strains

The fly strains used in this study include: (1) *acj6-Gal4* (Bourbon et al., 2002); (2) *tubP-LexA::GAD;FRT^{G13},UAS-mCD8,lexAop-rCD2::GFP/CyO,Y*; (3) *FRT^{G13},hs-FLP,tubP-Gal80/CyO,Y*; (4) *hs-FLP;FRT^{40A},UAS-rCD2::RFP,UAS-GFPi*; (5) *FRT^{40A},UAS-mCD8::GFP,UAS-rCD2i;TM3/TM6B*; (6) *actin-Gal4* (Bloomington stock #4414); (7) *y,w;FRT^{2A},FRT^{82B},96Ey⁺*; (8) *FRT^{2A},drone^{ΔA8}/TM6,Tb* (Kondo et al., 2006); (9) *hs-FLP,UAS-mCD8::GFP;FRT^{2A},Gal80;OK107*; (10) *FRT^{19A},hs-FLP,tubP-Gal80;Pin/CyO*; (11) *FRT^{19A},UAS-mCD8::GFP*; (12) *FRT^{19A},UAS-mCD8::GFP,N^{55e11}/FM7C*; (13) *FRT^{19A},hs-FLP,tubP-Gal80;Pin/CyO*; (14) *hs-FLP;FRT^{40A},numb¹/CyO*; (15) *y,w;FRT^{40A}/CyO,Y*; (16) *hs-FLP;FRT^{40A},tubP-Gal80/CyO*; (17) *FRT^{40A},Gal4-GH146,UAS-mCD8::GFP/CyO,Y*; (18) *FRT^{40A},numb¹,Gal4-GH146,UAS-mCD8::GFP/CyO,Y*; (19) *FRT^{40A},UAS-mCD8::GFP,UAS-rCD2i,numb¹,Gal4-GH146/CyO*; (20) *Gal4-MZ699/TM6B* (Ito et al., 1997); (21) *hs-FLP/CyO*; (22) *FRT^{19A}*; (23) *tubP-Gal4/TM3*; (24) *FRT^{19A},N^{55e11},Gal4-NP6115,UAS-mCD8::GFP/FM7C*; (25) *Gal4-GH298* (Stocker et al., 1990); (26) *Gal4-KL107* (Shang et al., 2007); (27) *asense-Gal4* (Zhu et al., 2006).

MARCM clonal analysis

Larvae with proper genotype were collected within 2 hours after hatching, heat shocked at 37°C for 1 hour to induce clones, and then cultured at 25°C until dissection at various desired stages. For twin-spot mosaic analysis with a repressible cell marker (MARCM) experiments, larvae were heat shocked at various stages at 37°C for 1 hour. When a widely expressed driver, such

as *asense-Gal4*, *actin-Gal4*, *tubP-Gal4* or *tubP-LexA::GAD*, was used to label MARCM clones, the duration of heat shock was shortened to 15 minutes to reduce unwanted background clones.

Immunohistochemistry and microscopy

Fly brains were dissected, stained and mounted as described in our previous study (Lee et al., 1999). Primary antibodies used in this study include rat anti-mCD8 mAb (1:100; Caltag), rabbit anti-GFP Ab (1:1000; Molecular Probes), mouse anti-Acj6 mAb (1:100; DSHB), mouse anti-nc82 mAb (1:100; DSHB), mouse anti-Elav (1:200; DSHB), mouse anti-rCD2 mAb (1:100; Serotec), rat anti-Dpn mAb (1:2) (Boone and Doe, 2008), rabbit anti-pH3 Ab (1:200; Upstate), rabbit anti-Dsred (1:500; Clontech) and rabbit anti-cleaved caspase-3 (1:200; Cell Signaling). FITC-, Cy3- and Cy5-conjugated secondary antibodies (Jackson ImmunoResearch) were used at the dilution of 1:200, 1:400 and 1:400, respectively. Immunofluorescent signals were collected by Zeiss LSM confocal microscope and processed using Osirix and Adobe Photoshop.

RESULTS

Production of adult adPNs occurs one by one, rather than in pairs

We must identify the sister neurons derived from an intermediate precursor, called a ganglion mother cell (GMC) in *Drosophila*, if we are to determine if fate diversification through asymmetric cell division occurs during the final neuron-producing mitoses. Using MARCM we can label the progeny of a GMC, typically two cells as a two-cell clone, following mitotic recombination in a dividing Nb and loss of the Gal4 repressor, Gal80, from the derived GMC (Lee and Luo, 1999). Alternatively, loss of Gal80 from the regenerated Nb would lead to labeling of the remaining lineage as a multicellular Nb clone. In the case of *Gal4-GH146*-labeled adPN clones, we could readily obtain adPN Nb clones following mitotic recombination at the first instar stage. But, instead of a two-cell clone, we consistently observed a single DL-1 neuron when an adPN Nb clone was not present ($n > 100$). Many adPNs are negative for *Gal4-GH146* (Lai et al., 2008) and so it is possible that the single DL-1 neuron has an unmarked sister adPN.

However, when adPN clones were marked with *acj6-Gal4* (Bourbon et al., 2002; Komiyama et al., 2003), a Gal4 driver known to label all the progeny of the adPN lineage (Fig. 1) (Lai et al., 2008), we did not obtain two-cell clones either (data not shown). This raised the possibility that mature adPNs arise one by one, rather than in pairs as in the well-characterized lineages of MB neurons (Lee et al., 1999). Direct demonstration of this requires confirmation of the sisterhood between Nb clones and the lone adPNs. A modification of MARCM, called twin-spot MARCM, allows one to label paired sister clones differentially with two reporters, *UAS-mCD8::GFP* and *UAS-rCD2::RFP*, at the same time (Yu et al., 2009). As to clones derived from Nbs, twin-spot MARCM permits direct identification of the GMC progeny that associates with a particular Nb clone (Fig. 2A). Use of the pan-adPN *acj6-Gal4* driver in twin-spot MARCM should allow us to unambiguously determine if the adPN Nb consistently deposits only one postmitotic neuron that persists into the adult stage at one time.

We induced low-frequency mitotic recombination via mild heat shock at different larval stages, resulting in adPN clones labeled with *acj6-Gal4*. Intriguingly, every adPN Nb clone we obtained was accompanied by a single adPN that was labeled differentially and should constitute the GMC side of the twin spots (Fig. 2B-G; $n = 20$). The pairing of multicellular Nb clones of various sizes with apparently single-cell clones substantiates the notion that every self-renewing asymmetric Nb division generates one adult PN.

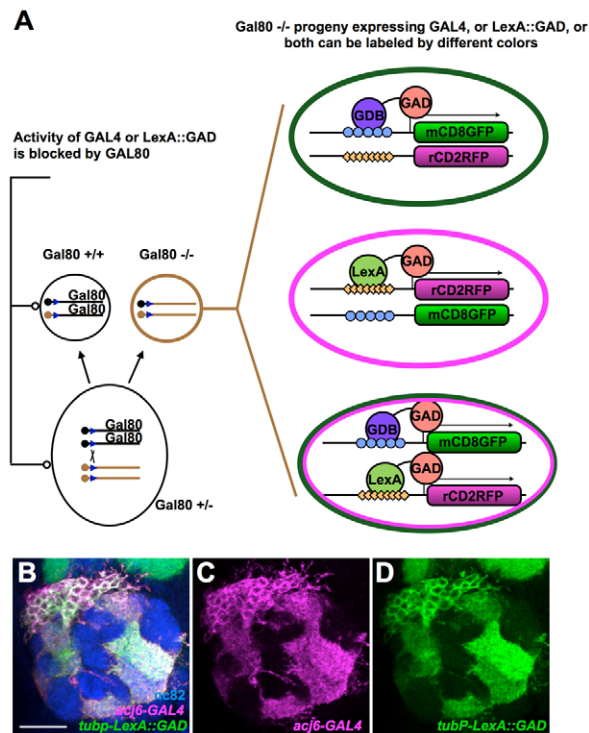


Fig. 1. *acj6-Gal4* labels the entire postembryonic adPN lineage.

(A) The principle of dual-expression control MARCM. Loss of *Gal80*, following mitotic recombination, de-represses *Gal4* and *LexA::GAD* such that *Gal80*-minus progeny positive for *Gal4*, *LexA::GAD*, or both can be differentially marked, as shown here in green, magenta or green plus magenta. (B–D) A *Gal80*-minus adPN Nb clone co-labeled with *tubP-LexA::GAD* (green) and *acj6-Gal4* (magenta). Note, on a single focal plane, that the entire clone, as revealed by *tubP-LexA::GAD*, was labeled in full with *acj6-Gal4*. The adult antennal lobe was counterstained with nc82 mAb (blue). Scale bar: 20 μ m.

Each adPN GMC makes an adPN and a mysterious sibling that is eliminated by programmed cell death

Meanwhile, we detected unpaired single-cell clones, especially following induction of mitotic recombination at mid-larval stages when most clones we obtained derived from GMCs (possibly owing to the presence of more GMCs per lineage at mid-larval stages). These solitary adPNs are probably derivatives of GMCs, but in great contrast with the progeny of MB GMCs, which consistently exist in pair when differentially marked by twin-spot MARCM (Yu et al., 2009). To further demonstrate presence of GMCs that make only one mature adPN despite active cell division, we sought to locate mitotic GMCs in the proliferating adPN Nb clones.

Two independent approaches were taken to identify the progenitors of adPNs in larval brains. First, in the larval brains doubly marked with *asense-Gal4* (a neural precursor driver) (Zhu et al., 2006) and *LexA::GAD-GH146* (Lai et al., 2008), we located adPN precursors by identifying the *Asense*-positive cluster with nascent projections that had merged into the bundle of GH146-positive adPN neurites (data not shown). Second, we labeled precursors as well as postmitotic neurons in any given MARCM clone using a ubiquitous *Gal4* driver, and determined the adPN Nb clones based on cell body positions and their characteristic neurite trajectories (e.g. Fig. 3A). Cells undergoing mitosis can be

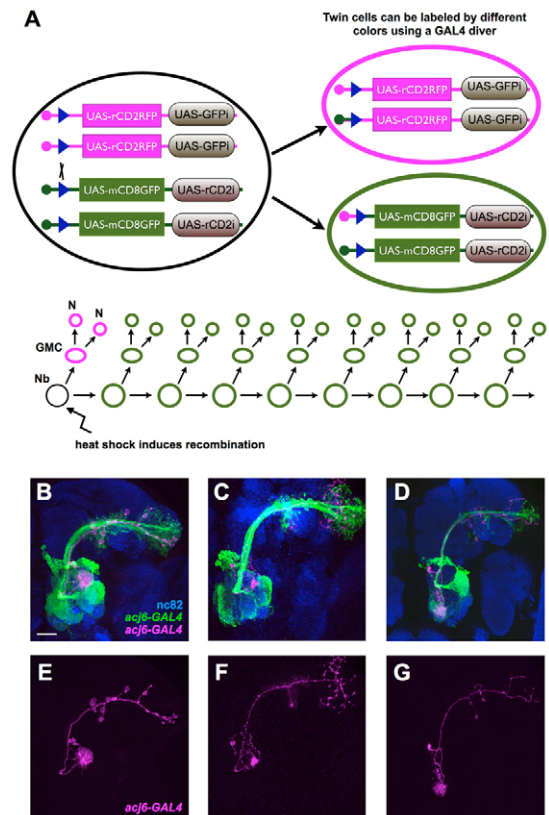


Fig. 2. The adPN Nb makes one PN at one time. (A) The principle of twin-spot MARCM. Following mitotic recombination, one homozygous daughter cell loses repressor 1 (miRNA against *rCD2*) and becomes specifically labeled with reporter 1 (*UAS-rCD2::RFP*), while the other daughter cell loses repressor 2 (miRNA against *GFP*) and expresses reporter 2 (*UAS-mCD8::GFP*). As to clones derived from a self-renewing Nb, one can label the GMC clone and the remaining lineage in different colors at the same time using twin-spot MARCM. (B–G) Merged confocal images of adult adPN twin-spot MARCM clones generated at early (B, E), middle (C, F), or late (D, G) larval stages. Nb clones of different sizes (green) were consistently accompanied by lone adPNs (magenta). Brain neuropils were counterstained with nc82 mAb (blue). Scale bar: 20 μ m.

identified with Phosphohistone H3 (pH3) antibody (Hendzel et al., 1997). In both cases, we could detect pH3-positive small cells that lie adjacent to the big Nb in about 70% of the adPN lineages (e.g. Fig. 3A). About 70% of adPN Nbs were positive for pH3 as well (data not shown). These observations demonstrate occurrence of mitosis in the immediate derivatives of adPN Nbs and substantiate the presence of intermediate precursors that possibly divide once more to make mature adPNs.

However, only one adPN persists from each pair of postmitotic cells into the adult stage (Fig. 2E–G), raising the possibility that the other sibling may die during development. Most neuronal lineages, including the adPN lineage, complete their proliferation by pupal formation (Ito and Hotta, 1992; Truman and Bate, 1988). Notably, the adPN Nb clones examined at the wandering larval stage carried a similar number of cells as the mature adPN Nb clones (compare Fig. 3C with 3B). This result suggests that the mysterious siblings have largely disappeared before pupation and possibly through apoptosis. To locate such mysterious siblings, we first examined if

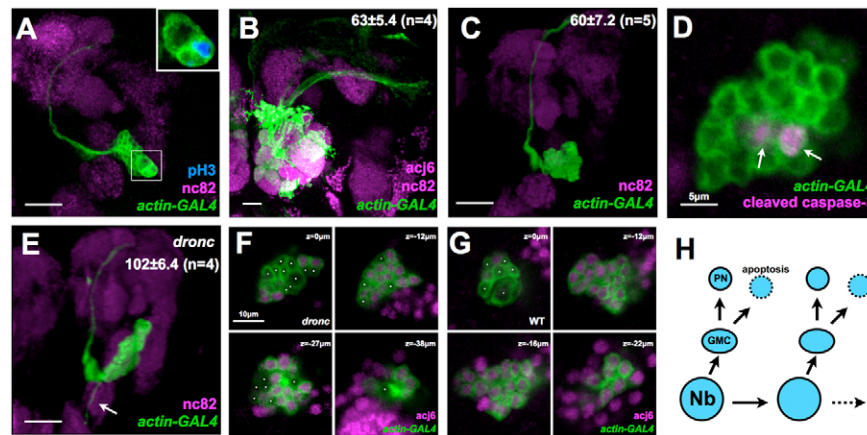


Fig. 3. Each adPN is born with a mysterious sibling that dies through apoptosis. (A) Presence of a pH3-positive GMC (blue) in a developing adPN Nb clone (green). The adPN clone was labeled with *actin-Gal4*, and the larval brain was co-immunostained with anti-pH3 Ab (blue) and nc82 mAb (magenta). (B,C) adPN Nb clones (green), induced around larval hatching, carried similar numbers of cells no matter whether they were examined in adults (B) or at the wandering larval stage (C). (D) Pro-apoptotic cells (arrows) were detected by anti-cleaved caspase-3 Ab (magenta) in a larval adPN Nb clone (green), as shown on a single focal plane of its cell body region. (E) Over 100 cells exist in *dronc* mutant adPN Nb clones that normally carry about 60 cells. The mutant clones also extend an ectopic bundle of neurites (arrow) that projects downward away from the iACT. (F,G) Four different confocal sections of *dronc* mutant larval adPN clones (F) or wild type (G) were labeled with *actin-Gal4* (green), and the larval brains were co-immunostained with anti-Acj6 Ab (magenta). z=how deep each confocal section was relative to the positions of the Nbs that locate close to the brain surface. Compared with wild-type adPN clones in which Acj6-negative cells could only be found near the Nbs, *dronc* mutant clones carry many more Acj6-negative cells, which distribute through the clones. Acj6-negative cells are indicated with white dots inside cell bodies. (H) A model of adPN neurogenesis. The Nb repeatedly generates GMCs that make one PN and a mysterious sibling through each asymmetric cell division. The mysterious siblings are eliminated during development by apoptosis. Scale bars: 20 μ m in A, C and E.

one could detect apoptotic cells in developing adPN clones. Cleavage of caspases triggers apoptosis, and the pre-apoptotic cells can be identified with antibodies that specifically recognize cleaved forms of caspases (Yu et al., 2002). Using the cleaved caspase-3 antibody, we readily found a small number of cells that exhibit high levels of cleaved caspase in most adPN Nb clones that were still proliferating (100%, $n=3$; e.g. Fig. 3D). Mutations in *dronc* (*Nedd2-like caspase* – FlyBase), which encodes the *Drosophila* initiator caspase, can effectively block apoptosis in diverse developmental contexts (Chew et al., 2004; Dorstyn et al., 1999; Kondo et al., 2006). To uncover the adPN mysterious siblings, we sought to block apoptosis in adPN Nb clones.

We generated *dronc* mutant adPN Nb clones and determined if more cells exist in the clones that were defective in apoptosis. Entire adPN Nb clones were labeled with *actin-Gal4* (Bloomington stock #4414; Y. Hiromi, unpublished), and could be identified at the wandering larval stage based on their progeny consisting of a specific group of Acj6-positive cells that are situated beside the larval antennal lobe (e.g. Fig. 3C,E). In addition, they consistently extend neurites through the inner antennocerebral tract (iACT) to the dorsal posterior brain region (Jefferis et al., 2001; Jefferis et al., 2004). Intriguingly, *dronc* mutant adPN Nb clones carried many more cells than their wild-type controls (102 ± 6.4 in Fig. 3E versus 60 ± 7.2 in Fig. 3C). About one-third of the *dronc* mutant offspring are negative for Acj6 (Fig. 3F). By contrast, only the newborn situated around the Nbs are negative for Acj6 in wild-type adPN clones (Fig. 3G). Mutant clones further acquired an ectopic bundle of neurites that project ventrally as the main bundle turns dorsally into the iACT (arrow in Fig. 3E). Blocking apoptosis apparently rescued the adPN mysterious sibs that, in contrast with adPNs, are mostly negative for Acj6 and may acquire a non-PN fate judging from their initial neurite trajectory.

These results indicate that each GMC of the adPN lineage makes an adPN and a mysterious sibling, potentially through asymmetric cell division, and that the non-PN half lineage is completely eliminated by programmed cell death (Fig. 3F). As reported in an accompanying paper (D. W. Williams and J. W. Truman), many lineages in the *Drosophila* ventral ganglion also exist as hemilineages. Blocking apoptosis analogously rescued their missing half progenies. Despite no net gain at the cell level, asymmetric cell division during final neuron-producing mitoses governs proper specification of many neuronal terminal fates.

Numb antagonizes Notch to specify PNs in the adPN lineage while Notch activity promotes PN fate in the vPN lineage

Numb antagonizes Notch signaling to guide development of sister cells along one or the other pre-specified path in diverse cases of asymmetric cell division (Bardin et al., 2004). If adPN GMCs had undergone asymmetric cell division to make progenies with distinct fates, we reasoned that knocking out *notch* or *numb* would transform adPNs to their mysterious siblings or vice versa. Consistent with these predictions, adPN Nb clones that were made homozygous for a *notch* mutation carried twice as many adPNs, which were otherwise grossly normal (Fig. 4A,B). We also regained typical two-cell clones of adPNs in *notch* mutant mosaic brains (16%, $n=100$; comparable to the 18% chance of getting Nb clones), as opposed to those single-cell-containing GMC clones in wild-type mosaic brains (Fig. 4C,D). The doubling of cell numbers in both Nb and GMC clones supports involvement of Notch-dependent asymmetric division in the derivation of only one mature neuron from each GMC of the adPN lineage.

By contrast, comparable induction of mitotic recombination failed to yield *numb* mutant Nb clones of adPNs in the brains that were apparently mosaic ($n=103$). In non-mosaic brains, bilaterally

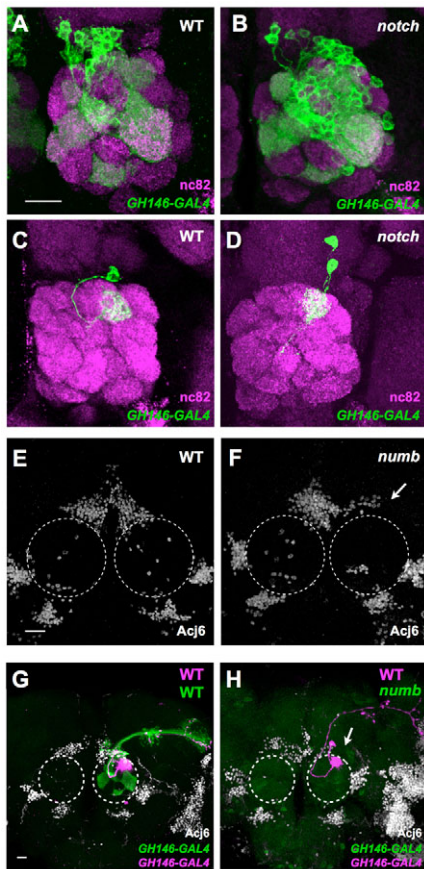


Fig. 4. Suppression of Notch by Numb promotes the PN fate in the adPN lineage. (A–D) Adult wild-type (A, C) and *notch*^{−/−} (B, D) adPN Nb (A, B) and GMC (C, D) clones, generated around larval hatching, were labeled by *Gal4-GH146* (green). Note doubling of the Nb cell numbers as well as appearance of two-cell clones in the absence of Notch (B, D). The antennal lobe glomeruli were revealed by counterstained with nc82 mAb (magenta). (E, F) Adult brains carrying wild-type (E) or *numb* mutant (F) adPN clones that were generated around larval hatching. Note the asymmetric loss of Acj6-positive adPNs in some *numb* mutant mosaic brains, as indicated by the arrow in F where a *numb* adPN Nb clone presumably exists in the right brain lobe. The dashed circles outline the antennal lobes. (G, H) Adult brains carrying wild-type (G) or *numb* mutant (H) adPN twin-spot MARCM clones that were generated around larval hatching. Note that in wild-type clones, magenta single-cell clones were always associated with green Nb clones (e.g. G; 100%, *n*=13). Acj6 staining labeled adPN neurons asymmetrically on the dorsal side of the antennal lobes (outlined by dashed circles). By contrast, the green *numb* mutant Nb clones were missing in the brain lobes carrying magenta wild-type GMC clones (e.g. H; 100%, *n*=22). The asymmetric loss of Acj6-positive adPNs was consistently detected on the same side as those unpaired GMC clones. Scale bars: 20 μ m. WT, wild type.

symmetric clusters of Acj6-positive cells exist around the antennal lobes (Fig. 4E). Many Acj6-positive cells in the dorsal cluster are descendants of the larval adPN Nb (Komiyama et al., 2003; Lai et al., 2008). Notably, unilateral reduction in the dorsal cluster size occurred in the *numb* mutant mosaic brains at a frequency (13.3%) comparable to the frequency of adPN Nb clones in controls (e.g. compare Fig. 4F with 4E). This observation suggests that whereas adPNs double in *notch* mutant Nb clones, adPNs are transformed to their mysterious siblings in *numb* mutant Nb clones. To substantiate

that *numb* mutant adPNs had been transformed to their mysterious siblings and thus vanished, we repeated the mosaic experiment with twin-spot MARCM to locate the wild-type sister clones of those missing *numb* mutant clones in the brains where the dorsal cluster of Acj6-positive cells were largely gone. Clone induction in newly hatched larvae consistently led to production of Nb clones paired with single-cell-containing GMC clones in wild-type mosaic brains (100%, *n*=13; e.g. Fig. 4G). By contrast, we obtained wild-type GMC clones at a comparable frequency, but they were unpaired in *numb* mutant mosaic brains. Furthermore, those unpaired GMC clones were present exclusively in the brain lobes that have lost most of the dorsally located Acj6-positive cells (100%, *n*=22; e.g. Fig. 4H). In sum, whereas adPNs double in *notch* mutant Nb clones, adPNs are transformed to their mysterious siblings in *numb* mutant Nb clones. These results indicate that suppression of Notch signaling by Numb specifies the PN cell fate in the adPN lineage.

Interestingly, opposite results were obtained in the vPN lineage. *Gal4-GH146* permits labeling of six vPNs in wild-type vPN Nb clones (Fig. 5A) (Jefferis et al., 2001). The GH146-positive vPNs doubled in *numb* mutant Nb clones but apparently disappeared in *notch* mutant mosaic brains (Fig. 5). Loss of vPNs upon depletion of Notch from their precursors was evident when we located vPNs with *Gal4-MZ699* (Lai et al., 2008) and without *tubP-Gal80* in *notch* mutant mosaic brains (compare Fig. 5D with 5C). These phenotypes suggest that the vPN lineage, like the adPN lineage, normally exists as a hemilineage. But Notch signaling, instead of promoting the mysterious sibling fate as in the adPN lineage, specifies the PN fate in the vPN lineage.

Notch on-or-off specifies non-PNs versus PNs in the IAL lineage

How about Notch/Numb and regulation of sibling neuronal cell fates in the more heterogeneous IAL lineage? Unlike the adPN or vPN lineage that homogeneously consists of PNs with similar trajectories, the IAL lineage yields diverse types of neurons, including a subset of GH146-positive PNs, atypical PNs, AL local interneurons (LNs), and even non-AL neurons (Lai et al., 2008). The IAL Nb, as in most protracted lineages, makes specific neuron types at specific developmental times (Lai et al., 2008). However, different types of IAL progeny, such as AL PNs and LNs, may be co-produced (Lai et al., 2008), raising the possibility that distinct IAL neurons are derived in pair through asymmetric cell division. This possibility is nicely supported by the observation that loss of Notch or Numb oppositely affects the GH146-positive IAL PNs. As in the adPN lineage, there were twice as many conventional PNs in *notch* mutant IAL Nb clones (Fig. 6A), and no GH146-positive IAL PNs could be detected in *numb* mutant mosaic brains (data not shown).

To confirm if Notch/Numb-dependent asymmetric cell division underlies co-production of distinct IAL neuron types, and to further determine the pairing relationships among the diverse types of IAL progeny, we characterized neuron type compositions in the IAL lineage that was rendered mutant for *notch* or *numb*. We have previously determined the neuron type compositions of the wild-type IAL lineage by clonal analysis with dual-expression-control MARCM (Lai et al., 2008). This genetic mosaic labeling technique allows one to mark a *Gal80*-minus clone using two independent binary transcriptional systems (Lai and Lee, 2006). A ubiquitous LexA::GAD driver permits detection of all *Gal80*-minus clones as well as visualization of every single cell in the clones. This reveals entire IAL clones (e.g. inset in Fig. 6C). In combination with a subtype-specific *Gal4*, one can unambiguously determine if any and how many of the progeny have acquired a particular cell fate.

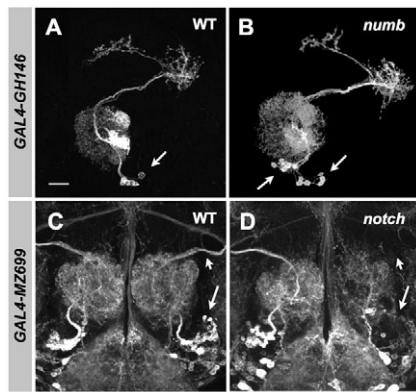


Fig. 5. Contrary to the adPN lineage, the vPN lineage requires Notch for specification of PNs via asymmetric cell division.

(A,B) Adult wild-type (A) and *numb*^{-/-} (B) vPN Nb clones, generated around larval hatching and labeled by *Gal4-GH146*. Note duplication of vPNs (arrows in B) in the *numb* mutant clone. (C,D) Adult brains carrying wild-type (C) or *notch* mutant (D) vPN clones that were generated around larval hatching. The vPN lineages (long arrow: cell body region; short arrow: neurite trajectory) were revealed by *Gal4-MZ699*-dependent expression of *UAS-mCD8::GFP*. Note the unilateral missing of the vPN lineage in some *notch* mutant mosaic brains (e.g. D). Scale bar: 20 μ m. WT, wild type.

We determined the changes in the neuron type composition of *notch* or *numb* mutant IAL Nb clones by dual-expression-control MARCM with *tubP-LexA::GAD* to visualize the entire IAL Nb clone, plus *Gal4-GH146*, *Gal4-NP6115*, *Gal4-GH298* or *Gal4-KL107* as cell-fate markers for uniglomerular PNs, atypical PNs, and various subtypes of AL LNs, respectively (Lai et al., 2008). In addition, we simultaneously located Acj6-positive IAL neurons, including additional types of LNs as well as non-AL neurons, by co-immunostaining with anti-Acj6 Ab. Notably, mutually exclusive types of neurons existed in *notch* versus *numb* mutant IAL Nb clones. IAL Nb clones mutant for *notch* carried excessive uniglomerular PNs and atypical PNs at the expense of various types of LNs and Acj6-positive neurons (Fig. 6A-D). Conversely, no PN existed in the *numb* mutant IAL Nb clones, as evidenced by missing of PN trajectories as well as cell-fate markers. Despite massive proliferation (see next section of Results), the over-sized *numb* mutant IAL Nb clones were composed of only LNs and Acj6-positive neurons (Fig. 6E,F). These phenotypes suggest that PNs and non-PNs of the IAL lineage are derived in pair through asymmetric cell division. It is evident that Notch on-or-off specifies non-PNs versus PNs during asymmetric cell division of IAL GMCs.

Lineage- and stage-specific effects of Numb on neuronal precursors

The overproliferation of *numb* mutant IAL Nb clones (Fig. 6E,F) suggests possible involvement of Numb in regulating neurogenesis, potentially through an action in GMCs, which exclusively inherit Numb proteins during self-renewal of Nbs (Doe, 2008; Knoblich, 2008). Two basally located proteins, Brat and Prospero, as well as the mechanisms that govern segregation of the basal complex into GMCs have been shown to confer proper GMC cell fate (Betschinger et al., 2006; Lee et al., 2006a). Besides, Numb and Brat are essential for the maturation of the transit amplifying neuronal precursors in the unconventional PAN (Posterior Asense-negative) lineages (Bello et al., 2008; Boone and Doe, 2008; Bowman et al.,

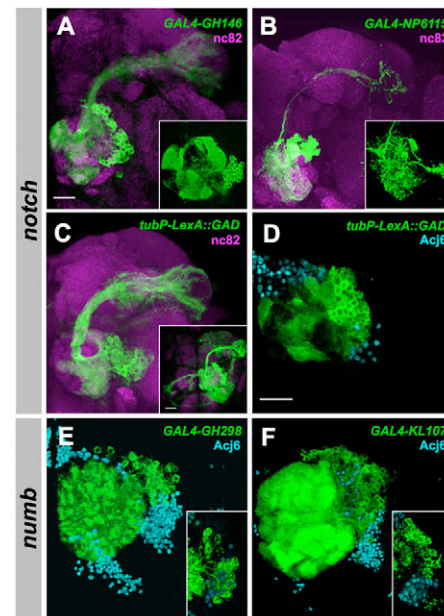


Fig. 6. Notch and Numb specify the non-PNs and the PNs respectively in the IAL lineage.

(A-F) IAL *notch*^{-/-} (A-D) or *numb*^{-/-} (E,F) Nb clones (green) labeled with *Gal4-GH146* (A), *Gal4-NP6115* (B), *tubP-LexA::GAD* (C,D), *Gal4-GH298* (E) or *Gal4-KL107* (F). Neuropils were counterstained with nc82 mAb (magenta), and Acj6-positive cell bodies were detected with anti-Acj6 Ab (cyan). Close-up views of the AL regions were shown in D-F. As compared to the wild-type controls (insets in A to C: insets in A and B show the AL regions only, and inset in C shows an entire IAL clone; note that in C the interneuron-like processes on the dorsal part of the right hemisphere is a background clone), *notch* mutant IAL Nb clones carry supernumerary GH146-positive PNs (A) and more NP6115-positive atypical PNs (B) at the expense of AL interneurons (lack of interneuron innervation patterns in C) and Acj6-positive cells (very few Acj6-positive cells within the clone in D). By contrast, PNs of various types were apparently transformed to GH298- or KL107-positive interneurons as well as to diverse types of Acj6-positive cells in *numb* mutant IAL Nb clones (E,F). The cell numbers of the non-PN types are drastically increased in E and F, compared with those of their wild-type controls (insets in E and F). Scale bars: 20 μ m in A,D; 40 μ m in C, inset.

2008). PAN Nb clones lacking Numb or Brat yield many transit amplifying precursors that proliferate without maturation and fail to make any postmitotic neuron (Bowman et al., 2008). By contrast, *numb* mutant IAL clones carried numerous differentiated neurons in addition to multiple Nbs (Fig. 6E,F). This possibly reflects roles of Numb in regulating typical neurogenesis (Lee et al., 2006b; Wang et al., 2006).

To probe such lineage-specific Numb functions in neurogenesis, we examined developing Nb clones and detected three types of *numb* mutant Nb clones. Clones were induced at the newly hatched larval stage, and examined at the mid-third instar larval stage when Nbs were still actively dividing. We obtained comparable numbers of clones in wild-type versus *numb* mutant mosaic brains (Fig. 7A). However, in contrast with the control that consistently exhibits one Nb per clone, *numb* mutant Nb clones may carry multiple large cells or many more intermediate precursors. We first noticed presence of mutant PAN Nb clones that carried numerous immature transit amplifying precursors possessing small cell bodies positive for Deadpan and negative for Elav (Fig. 7C). Close inspection of the less

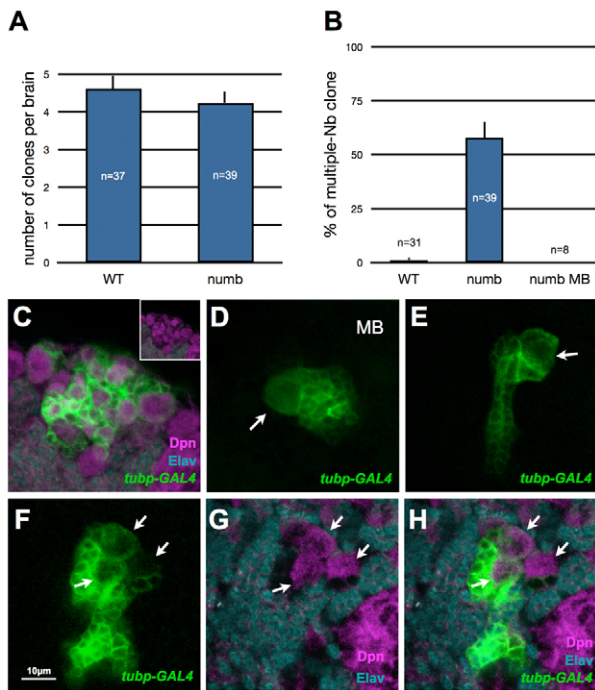


Fig. 7. Presence of ectopic Nbs in certain *numb*^{-/-} Nb clones.

(A) The average number of wild-type or *numb*^{-/-} Nb clones that were obtained per brain of the mid-third instar larvae, following a 15-minute heat shock at 37°C at the newly hatched larval stage. (B) The percentage of wild-type, *numb*^{-/-} or *numb*^{-/-} MB Nb clones that contain multiple Nbs. (C) A *numb*^{-/-} Nb clone contains only Dpn-positive precursors without mature neurons. The mosaic brain was triply labeled with *tubP-Gal4*-dependent expression of *UAS-mCD8::GFP* (green), anti-Dpn Ab (magenta), and anti-Elav Ab (cyan); the inset is the same image without showing *tubP-Gal4* expression. (D,E) A *numb*^{-/-} MB clone (D) and a *numb*^{-/-} non-MB Nb clone (E) that both carry only one Nb per clone, as revealed by the presence of only one big cell (arrows) in the entire clones marked by *tubP-Gal4* (green). (F-H) Single confocal images of a multi-Nb *numb*^{-/-} Nb clone that was labeled with *tubP-Gal4* to outline the entire clone (green) and immunostained with anti-Dpn and anti-Elav Abs to highlight Nbs (magenta) and postmitotic neurons (cyan), respectively. Note the presence of multiple Dpn-positive big cells (arrows) in the clone. WT, wild type.

prominent non-PAN Nb clones further allowed us to reveal *numb* mutant Nb clones with two distinct phenotypes. About 50% of the collected non-PAN Nb clones, including all *numb* mutant MB Nb clones, were grossly normal (Fig. 7A,B,D,E). In contrast, we observed multiple Nbs, as evidenced by the Deadpan-positive large cell bodies, per clone in the other 50% of the *numb* mutant non-PAN Nb clones (Fig. 7A,B,F-H). Unlike *brat* or *lgl* [*l(2)gl* – FlyBase] mutant Nb clones that are packed with Nb-like cells (Betschinger et al., 2006; Lee et al., 2006a), the *numb* mutant multi-Nb clones mostly carried two or three Nbs and consistently made normal-looking GMCs as well as Elav-positive postmitotic neurons (Fig. 7F-H). It appears that loss of Numb in certain lineages causes mild or transient defects in the specification of GMC versus Nb. This is consistent with a recent publication that mutations in protein phosphatase 2A, a Numb regulator, caused mild Nb overproliferation in non-PAN lineages (Wang et al., 2009). Having one or two ectopic Nbs could nicely explain why *numb* mutant IAL Nb clones can produce two to three folds of neurons with the Notch-on sibling cell fates (Fig. 6E,F).

These observations suggest lineage-dependent Notch/Numb functions in the regulation of neural precursor self-renewal as well as the specification of sibling cell fates.

DISCUSSION

In contrast to MB lineages, in which GMCs divide to make two indistinguishable neurons (Lee et al., 1999), the three AL neuronal lineages we examined here produce GMCs that consistently undergo asymmetric cell division and yield daughter cells with distinct fates. This mechanism allows doubling of neuron types, as in the IAL lineage. However, in the adPN and vPN lineages, only one from each pair of daughter cells persists into the adult stage. They are both present as hemilineages. Notably, about 50% of central brain lineages exist as hemilineages, as revealed by clonal analysis with twin-spot MARCM using a pan-neuronal driver (Yu et al., 2009). Recovery of the missing hemilineages in the *Drosophila* VNC has implicated the Notch/Numb-mediated asymmetric cell division as a mechanism for divergent configuration of distinct insect brains (D. W. Williams and J. W. Truman, unpublished). In sum, asymmetric cell division is broadly utilized; depending on the lineages, a GMC may divide to make two identical neurons, two distinct neurons, or only one mature neuron.

Notch and Numb underlie asymmetric cell division in diverse contexts, including the asymmetric cell divisions of diverse AL PN precursors. Notably, the output of Notch signaling is grossly opposite in the adPN versus vPN lineage. Each GMC in both lineages makes one PN and one mysterious sibling. Interestingly, Notch-on specifies the PN fate in the vPN lineage but antagonizes the PN fate in the adPN lineage (Figs 4, 5). The cell-fate determinants for PNs of different lineages could be more distinct than their gross phenotypes suggest. In addition, the mysterious siblings of adPNs versus vPNs, upon rescued, might acquire very different fates. These lineage-dependent outputs of Notch signaling support the argument for its involvement in modulating cell differentiation, rather than specifying any de novo cell fate. It appears that two, possibly mutually exclusive, cell fates pre-exist in each precursor, and that Notch signaling, which occurs only in Numb-negative daughter cells, triggers cell differentiation along one rather than the other pre-programmed path.

Notch/Numb-dependent asymmetric cell division underlies the derivation of two complex IAL hemilineages that both persist into the adult stage. Distinct PN types are made along the Notch-off hemilineage, whereas diverse types of non-PNs, including various AL LNs and most Acj6-positive progeny, differentiate from Numb-negative daughter cells (Fig. 6). As in other neuronal lineages, specific neuron types of the IAL lineage are made at specific times of development (Lai et al., 2008). However, it remains uncertain whether specific PN types consistently pair with specific non-PN types through the production of the sister hemilineages. Superficially, there exist many more non-PN types than the recognizable PN types in the IAL lineage, raising the possibility that neuronal temporal identity is altered in distinct paces between the two IAL hemilineages. Determining individual IAL GMCs and their derivatives is essential for resolving the detail and further elucidating how two parallel sets of temporal cell fates can be generated by a common progenitor through repeated self-renewal.

Besides governing neuronal cell fates following asymmetric cell division of GMCs, Numb, together with other basal complex proteins, including Brat and Prospero, is selectively segregated into GMCs during self-renewal of Nbs (Doe, 2008; Knoblich, 2008). However, in contrast with its essential role for preventing the transit-amplifying precursors from undergoing tumor-like overproliferation

in PAN lineages (Bowman et al., 2008), the function of Numb in restraining the basally situated Nb offspring from adopting Nb fate varies among non-PAN lineages and depends on the stage of development. Notably, Numb is required in certain non-PAN neuronal lineages, including the IAL lineage, for preventing production of ectopic Nbs (Fig. 7). Although Notch is dispensable for maintaining the stem cell fate in IAL Nbs, it remains likely that loss of Numb leads to ectopic Notch signaling, which in turn promotes stem cell fate in otherwise GMCs. The differential requirement of Numb for proper specification of GMCs of different origins could be due to lineage- and/or stage-dependent variations in the abundance of Notch signaling components. Interestingly, the ectopic Nbs apparently maintain proper temporal identity and could make diverse neuron types as the endogenous progenitor. These raise the possibility that dynamic Notch signaling might be utilized in vivo to promote self-renewal versus amplification of Nbs.

Taken together, most neuron types in the *Drosophila* central brain are specified not only according to their lineage origin as well as birth order, but also depending on whether Numb exists to suppress Notch signaling in newly derived postmitotic neurons. It appears that postmitotic neurons are born with two opposing cell fates that were pre-determined in their immediate precursors based on their lineage and temporal origin. Notch signaling then suppresses the otherwise dominating fate. In addition, in certain neuronal lineages, Numb plays a subtle role in ensuring production of GMCs while Nbs undergo self-renewal. A conserved Notch/Numb-dependent mechanism probably governs diverse neural developmental processes through evolution.

Acknowledgements

We thank J. W. Truman and D. W. Williams for *dronc* mutant and sharing unpublished observations; C. Q. Doe for anti-Deadpan antibody; and C. T. Zugates for thoughtful comments on this manuscript. This work was supported by grants from National Institutes of Health (NS42049 and MH80739 to T.L.; DC005982 to L.L.). Deposited in PMC for release after 12 months.

References

- Bailey, A. M. and Posakony, J. W. (1995). Suppressor of hairless directly activates transcription of enhancer of split complex genes in response to Notch receptor activity. *Genes Dev.* **9**, 2609-2622.
- Bardin, A. J., Le Borgne, R. and Schweisguth, F. (2004). Asymmetric localization and function of cell-fate determinants: a fly's view. *Curr. Opin. Neurobiol.* **14**, 6-14.
- Bello, B. C., Izergina, N., Caussinus, E. and Reichert, H. (2008). Amplification of neural stem cell proliferation by intermediate progenitor cells in *Drosophila* brain development. *Neural Dev.* **3**, 5.
- Betschinger, J., Mechtler, K. and Knoblich, J. A. (2006). Asymmetric segregation of the tumor suppressor Brat regulates self-renewal in *Drosophila* neural stem cells. *Cell* **124**, 1241-1253.
- Boone, J. Q. and Doe, C. Q. (2008). Identification of *Drosophila* type II neuroblast lineages containing transit amplifying ganglion mother cells. *Dev. Neurobiol.* **68**, 1185-1195.
- Bourbon, H. M., Gonzy-Treboul, G., Peronnet, F., Alin, M. F., Ardourel, C., Benassayag, C., Cribbs, D., Deutsch, J., Ferrer, P., Haenlin, M. et al. (2002). A P-insertion screen identifying novel X-linked essential genes in *Drosophila*. *Mech. Dev.* **110**, 71-83.
- Bowman, S. K., Rolland, V., Betschinger, J., Kinsey, K. A., Emery, G. and Knoblich, J. A. (2008). The tumor suppressors Brat and Numb regulate transit-amplifying neuroblast lineages in *Drosophila*. *Dev. Cell* **14**, 535-546.
- Briscoe, J., Pierani, A., Jessell, T. M. and Ericson, J. (2000). A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* **101**, 435-445.
- Buescher, M., Yeo, S. L., Udolph, G., Zavortink, M., Yang, X., Tear, G. and Chia, W. (1998). Binary sibling neuronal cell fate decisions in the *Drosophila* embryonic central nervous system are nonstochastic and require inscuteable-mediated asymmetry of ganglion mother cells. *Genes Dev.* **12**, 1858-1870.
- Chew, S. K., Akdemir, F., Chen, P., Lu, W.-J., Mills, K., Daish, T., Kumar, S., Rodrigues, A. and Abrams, J. M. (2004). The apical caspase *dronc* governs programmed and unprogrammed cell death in *Drosophila*. *Dev. Cell* **7**, 897-907.
- Doe, C. Q. (2008). Neural stem cells: balancing self-renewal with differentiation. *Development* **135**, 1575-1587.
- Dorstyn, L., Colussi, P. A., Quinn, L. M., Richardson, H. and Kumar, S. (1999). DRONC, an ecdysone-inducible *Drosophila* caspase. *Proc. Natl. Acad. Sci. USA* **96**, 4307-4312.
- Endo, K., Aoki, T., Yoda, Y., Kimura, K. and Hama, C. (2007). Notch signal organizes the *Drosophila* olfactory circuitry by diversifying the sensory neuronal lineages. *Nat. Neurosci.* **10**, 153-160.
- Guo, M., Jan, L. Y. and Jan, Y. N. (1996). Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. *Neuron* **17**, 27-41.
- Hendzel, M. J., Wei, Y., Mancini, M. A., Van Hooser, A., Ranalli, T., Brinkley, B. R., Bazett-Jones, D. P. and Allis, C. D. (1997). Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromosoma* **106**, 348-360.
- Huttenre, A. and Knoblich, J. A. (2005). Numb and alpha-Adaptin regulates Sanpodo endocytosis to specify cell fate in *Drosophila* external sensory organs. *EMBO Rep.* **6**, 836-842.
- Ito, K. and Hotta, Y. (1992). Proliferation pattern of postembryonic neuroblasts in the brain of *Drosophila melanogaster*. *Dev. Biol.* **149**, 134-148.
- Ito, K., Sass, H., Urban, J., Hofbauer, A. and Schneuwly, S. (1997). GAL4-responsive UAS-tau as a tool for studying the anatomy and development of the *Drosophila* central nervous system. *Cell Tissue Res.* **290**, 1-10.
- Jan, Y. N. and Jan, L. Y. (2001). Asymmetric cell division in the *Drosophila* nervous system. *Nat. Rev. Neurosci.* **2**, 772-779.
- Jefferis, G. S., Marin, E. C., Stocker, R. F. and Luo, L. (2001). Target neuron prespecification in the olfactory map of *Drosophila*. *Nature* **414**, 204-208.
- Jefferis, G. S., Vyas, R. M., Berdnik, D., Ramaekers, A., Stocker, R. F., Tanaka, N. K., Ito, K. and Luo, L. (2004). Developmental origin of wiring specificity in the olfactory system of *Drosophila*. *Development* **131**, 117-130.
- Karcavich, R. and Doe, C. Q. (2005). *Drosophila* neuroblast 7-3 cell lineage: a model system for studying programmed cell death, Notch/Numb signaling, and sequential specification of ganglion mother cell identity. *J. Comp. Neurol.* **481**, 240-251.
- Kimura, Y., Satou, C. and Higashijima, S. (2008). V2a and V2b neurons are generated by the final divisions of pair-producing progenitors in the zebrafish spinal cord. *Development* **135**, 3001-3005.
- Knoblich, J. A. (2008). Mechanisms of asymmetric stem cell division. *Cell* **132**, 583-597.
- Knoblich, J. A., Jan, L. Y. and Jan, Y. N. (1995). Asymmetric segregation of Numb and Prospero during cell division. *Nature* **377**, 624-627.
- Komiyama, T., Johnson, W. A., Luo, L. and Jefferis, G. S. (2003). From lineage to wiring specificity. POU domain transcription factors control precise connections of *Drosophila* olfactory projection neurons. *Cell* **112**, 157-167.
- Kondo, S., Senoo-Matsuda, N., Hiromi, Y. and Miura, M. (2006). DRONC coordinates cell death and compensatory proliferation. *Mol. Cell. Biol.* **26**, 7258-7268.
- Lai, S. L. and Lee, T. (2006). Genetic mosaic with dual binary transcriptional systems in *Drosophila*. *Nat. Neurosci.* **9**, 703-709.
- Lai, S. L., Awasaki, T., Ito, K. and Lee, T. (2008). Clonal analysis of *Drosophila* antennal lobe neurons: diverse neuronal architectures in the lateral neuroblast lineage. *Development* **135**, 2883-2893.
- Lecourtis, M. and Schweisguth, F. (1998). Indirect evidence for Delta-dependent intracellular processing of notch in *Drosophila* embryos. *Curr. Biol.* **8**, 771-774.
- Lee, C. Y., Robinson, K. J. and Doe, C. Q. (2006a). Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. *Nature* **439**, 594-598.
- Lee, C. Y., Andersen, R. O., Cabernard, C., Manning, L., Tran, K. D., Lanskey, M. J., Bashirullah, A. and Doe, C. Q. (2006b). *Drosophila* Aurora-A kinase inhibits neuroblast self-renewal by regulating aPKC/Numb cortical polarity and spindle orientation. *Genes Dev.* **20**, 3464-3474.
- Lee, T. and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* **22**, 451-461.
- Lee, T., Lee, A. and Luo, L. (1999). Development of the *Drosophila* mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. *Development* **126**, 4065-4076.
- Marin, E. C., Jefferis, G. S., Komiyama, T., Zhu, H. and Luo, L. (2002). Representation of the glomerular olfactory map in the *Drosophila* brain. *Cell* **109**, 243-255.
- O'Connor-Giles, K. M. and Skeath, J. B. (2003). Numb inhibits membrane localization of Sanpodo, a four-pass transmembrane protein, to promote asymmetric divisions in *Drosophila*. *Dev. Cell* **5**, 231-243.
- Pearson, B. J. and Doe, C. Q. (2004). Specification of temporal identity in the developing nervous system. *Annu. Rev. Cell Dev. Biol.* **20**, 619-647.
- Rhyu, M. S., Jan, L. Y. and Jan, Y. N. (1994). Asymmetric distribution of Numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell* **76**, 477-491.
- Schweisguth, F. (2004). Notch signaling activity. *Curr. Biol.* **14**, R129-R138.
- Shang, Y., Claridge-Chang, A., Sjulson, L., Pypaert, M. and Miesenböck, G. (2007). Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe. *Cell* **128**, 601-612.

- Skeath, J. B. and Doe, C. Q.** (1998). Sanpodo and Notch act in opposition to Numb to distinguish sibling neuron fates in the *Drosophila* CNS. *Development* **125**, 1857-1865.
- Spana, E. P. and Doe, C. Q.** (1995). The Prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*. *Development* **121**, 3187-3195.
- Spana, E. P. and Doe, C. Q.** (1996). Numb antagonizes Notch signaling to specify sibling neuron cell fates. *Neuron* **17**, 21-26.
- Spana, E. P., Kopczynski, C., Goodman, C. S. and Doe, C. Q.** (1995). Asymmetric localization of Numb autonomously determines sibling neuron identity in the *Drosophila* CNS. *Development* **121**, 3489-3494.
- Stocker, R. F., Lienhard, M. C., Borst, A. and Fischbach, K. F.** (1990). Neuronal architecture of the antennal lobe in *Drosophila melanogaster*. *Cell Tissue Res.* **262**, 9-34.
- Struhl, G. and Adachi, A.** (1998). Nuclear access and action of Notch in vivo. *Cell* **93**, 649-660.
- Truman, J. W. and Bate, M.** (1988). Spatial and temporal patterns of neurogenesis in the central nervous system of *Drosophila melanogaster*. *Dev. Biol.* **125**, 145-157.
- Urbach, R. and Technau, G. M.** (2003). Segment polarity and DV patterning gene expression reveals segmental organization of the *Drosophila* brain. *Development* **130**, 3607-3620.
- Wang, C., Chang, K. C., Somers, G., Virshup, D., Ang, B. T., Tang, C., Yu, F. and Wang, H.** (2009). Protein phosphatase 2A regulates self-renewal of *Drosophila* neural stem cells. *Development* **136**, 2287-2296.
- Wang, H., Somers, W. G., Bashirullah, A., Heberlein, U., Yu, F. and Chia, W.** (2006). Aurora-A acts as a tumor suppressor and regulates self-renewal of *Drosophila* neuroblasts. *Genes Dev.* **20**, 3453-3463.
- Wheeler, S. R., Stagg, S. B. and Crews, S. T.** (2008). Multiple Notch signaling events control *Drosophila* midline neurogenesis, gliogenesis, and neuronal identity. *Development* **135**, 3071-3079.
- Wong, A. M., Wang, J. W. and Axel, R.** (2002). Spatial representation of the glomerular map in the *Drosophila* protocerebrum. *Cell* **109**, 229-241.
- Yu, H. H. and Lee, T.** (2007). Neuronal temporal identity in post-embryonic *Drosophila* brain. *Trends Neurosci.* **30**, 520-526.
- Yu, H. H., Chen, C. H., Shi, L., Huang, Y. and Lee, T.** (2009). Twin-spot MARCM to reveal the developmental origin and identity of neurons. *Nat. Neurosci.* **12**, 947-953.
- Yu, S.-Y., Yoo, S. J., Yang, L., Zapata, C., Srinivasan, A., Hay, B. A. and Baker, N. E.** (2002). A pathway of signals regulating effector and initiator caspases in the developing *Drosophila* eye. *Development* **129**, 3269-3278.
- Zeng, C., Younger-Shepherd, S., Jan, L. Y. and Jan, Y. N.** (1998). Delta and Serrate are redundant Notch ligands required for asymmetric cell divisions within the *Drosophila* sensory organ lineage. *Genes Dev.* **12**, 1086-1091.
- Zhu, S., Chiang, A.-S. and Lee, T.** (2003). Development of the *Drosophila* mushroom bodies: elaboration, remodeling, and spatial organization of dendrites in the calyx. *Development* **130**, 2603-2610.
- Zhu, S., Lin, S., Kao, C. F., Awasaki, T., Chiang, A. S. and Lee, T.** (2006). Gradients of the *Drosophila* Chinmo BTB-zinc finger protein govern neuronal temporal identity. *Cell* **127**, 409-422.